The growth of *Aeromonas hydrophila* K144 in ground pork at 5 °C

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The influence of NaCl, pH, atmosphere, and background microflora on the growth and/or survival of *Aeromonas hydrophila* K144 was studied in ground pork held at 5 °C. In ground pork, *A hydrophila* was sensitive to pH values below 6.0 in the form of either a low starting pH in the pork itself or induced by lactic acid bacteria action on added glucose. Growth of the organism is inhibited by NaCl levels of 3% (w/w) (approx. 4% brine content). *A. hydrophila* grew in vacuum-packaged ground pork; its growth was diminished by the presence of the naturally occurring meat microflora. Except for pH values below 6.0, conditions which inhibited growth permitted survival of the organisms for extended periods. Data indicate that the growth of *A. hydrophila* in ground pork can be controlled by factors such as NaCl, pH, and background microflora. In general, measures designed to control other foodborne pathogens appear adequate to limit *A. hydrophila.*

Key words: Psychrotrophic pathogen; Sodium chloride percentage; Brine; pH; Vacuum packaging; Mycroflora, competing

Introduction

Aeromonas hydrophila is a well-known cause of disease in fish, amphibia, and reptiles (Austin and Allen-Austin, 1985). It is also isolated commonly from cases of human diarrheal and non-gastrointestinal ailments in both immunocompetent and immunosuppressed patients, and is recognized increasingly as a human pathogen (Freij, 1986; Goodwin et al., 1983; Holmberg and Farmer, 1984; Ljungh and Wadstrom, 1985). While the bacterium is most commonly associated with water supplies, it has been hypothesized that foodborne dissemination may play a major role in the transmission of this suspected pathogen (Buchanan and Palumbo, 1985). *A. hydrophila* can be found as a part of the flora of refrigerated fresh meats, fish and poultry held under various conditions (Ayres, 1960; Blickstad and Molin, 1983; Eddy and Kitchell, 1959; Enfors et al., 1979; Gram et al., 1987; Grau et al., 1985; Hanna et al., 1977; Jay, 1967; Lee et al., 1985; Nagel et al., 1960; Palumbo et al., 1985a; Seideman et al., 1976; Simard et al., 1984) and is one of a growing list of

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potential pathogens which are capable of growth in foods held at refrigeration temperatures (Palumbo, 1986).

Recent surveys of retail fresh foods of animal origin determined that the organism was present in virtually every sample of fish and seafood, red meat, poultry, and raw milk examined (Palumbo et al., 1985a; Stern et al., 1987). A survey of retail vegetables also indicated a widespread occurrence of the organism in these products. One aspect of these surveys determined that the count of *A. hydrophila* increased in most foods during storage at 5 °C. In the survey of animal products (Palumbo et al., 1985a), fresh sausage (breakfast, Italian) was one exception to this observation. At the time, this was attributed to the salt content of fresh sausage, 1.5–2% NaCl (Kramlich et al., 1973). A second exception was the decrease of viable *A. hydrophila* in raw shucked oysters during one week's storage at 5 °C. The low pH of the sample (5.5) produced by a large population of lactic acid bacteria serves as a plausible explanation. There are no other data on the behavior of *A. hydrophila* in food systems, and especially on the effect of NaCl and other additives and packaging atmosphere on the response of *A. hydrophila.*

The purpose of this paper was to gain information on these parameters and the growth and/or survival of *A. hydrophila* in ground pork held at 5 °C.

Materials and Methods

Organism

Aeromonas hydrophila K144, a clinical isolate originally obtained from A. Ljungh (Palumbo et al., 1985b), was used in these studies. The organisms was grown in tryptic soy broth (Difco; 100 ml/500 ml flask) at 28 °C with shaking (200 rpm) overnight. The culture was then diluted in 0.1% peptone water as needed and added to the ground pork to yield a starting count of approx. 10^3/g, determined as described below.

Meat

Fresh picnics, hams, or pork shoulders were purchased as needed from a local supermarket. After purchase, the center of the meat was carefully boned and trimmed to minimize contamination and yield a product with a relatively low background count (approx. 10^3/g). After boning and trimming, the meat was ground through a sterile grinder [3/8 inch plate (0.95 cm)], the coarse meat inoculated with the diluted *A. hydrophila* culture, and additives (NaCl, glucose) mixed in and the mixture reground through a sterile grinder with a 1/8 inch plate (0.32 cm).

Variables

NaCl and glucose were added as described in the various experiments. In one series, the ground pork was irradiated (150 krad) to reduce the background flora of

the pork. After inoculation, the ground pork mixtures were held in sterile Stomacher bags at 5 °C and sampled at intervals. Anaerobic packages (packed in IKD All-Vak No. 13 oxygen permeability 1 cc/100 inch^2 (0.065 m^2)/24 at 77 °F (25 °C) [760 mm difference in pressure]; thickness, 0.003 inch (0.0076 cm); Kenfield Co.) were prepared with a Kenfield Vacuum sealer (model C 14). When sampling vacuum packed ground pork, the packages were examined visually and those which had lost vacuum were discarded.

Bacteriology

Aeromonas hydrophila. Viable counts of *A. hydrophila* were done by surface plating appropriate dilutions (made in 0.1% peptone water) onto starch ampicillin agar (Palumbo et al., 1985a). Plates were observed after 24 h at 28 °C; at that time, approx. 5 ml of Lugol's iodine was added to each plate and typical colonies (honey colored surrounded by a zone of starch hydrolysis) were counted as *A. hydrophila*.

Lactic acid bacteria and total aerobic count. In some experiments, lactic acid bacteria were counted by surface plating dilutions onto Rogosa SL agar (Difco); plates were counted after 3 days at 20 °C. Total aerobic flora was determined by surface plating appropriate dilutions onto APT agar (Difco) and incubating at 20 °C. Colonies were counted after 2 days.

pH and % brine. The brine content of the ground pork mixtures was calculated by the following formula:

$$\% \text{ brine} = \frac{\% \text{ NaCl}}{\% \text{ NaCl} + \% \text{ moisture}}$$

The % moisture was determined following overnight drying of the ground pork mixtures at 100 °C. The pH of the ground pork mixtures was measured by a combination electrode inserted into a 1:10 (w/v) dilution of the ground pork mixtures in distilled water.

Data. Data presented are from individual experiments. All variables were replicated at least twice and similar patterns were observed.

Results and Discussion

In the first series of experiments, the influence of NaCl and pH on the growth of *A. hydrophila* was studied (Fig. 1a and b). The experiment presented in Fig. 1a (top) utilized ground pork with a low starting pH, 5.90. At this low pH, 1% NaCl inhibited *A. hydrophila*; in this part of the study, growth of the control (Fig. 1a) was relatively weak, compare Fig. 1a with Fig. 1b (bottom). It was observed previously (Palumbo et al., 1985b) that *A. hydrophila* became more sensitive to pH and NaCl at low temperatures in broth cultures. These data in the ground pork system support the previous observation in broth.

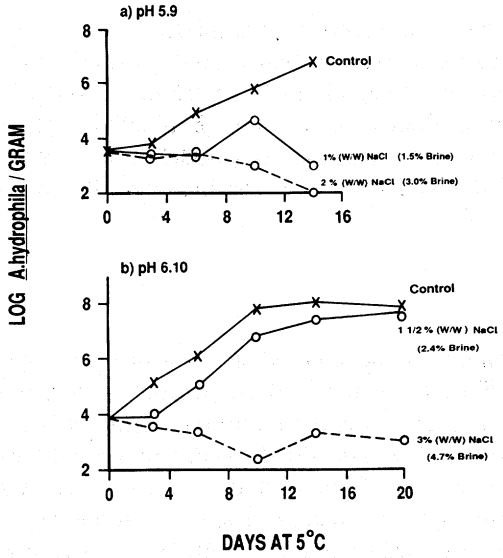

Fig. 1. Effect of NaCl and starting pH on the growth of *A. hydrophila* K144 in ground pork held at 5 ° C.

As indicated, the results of our food survey (Palumbo et al., 1985a) suggested that low counts of *A. hydrophila* in certain foods might be due to low pH of the food. It can be seen above that salt sensitivity increases at the low pH value. The effect of pH itself was studied (Fig. 2). In this experiment, 1% glucose was added to the ground pork and the count of viable *A. hydrophila* and the pH were followed during refrigerated storage. It was observed that during the early stages of storage, the organism behaved similarly to the control in Fig. 1b; then, as the pH of the pork

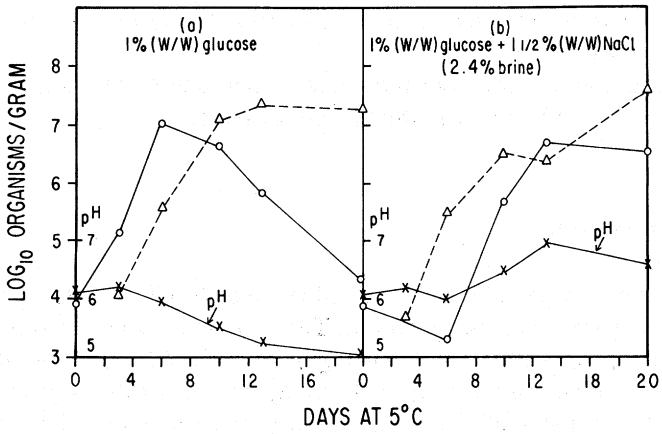

Fig. 2. Effect of NaCl (1.5 (w/w) NaCl, 2.4% brine) on the response of *A. hydrophila* K144 in ground pork containing 1% (w/w) glucose and held at 5 ° C (○, count of *A. hydrophila;* △ count of lactic acid bacteria).

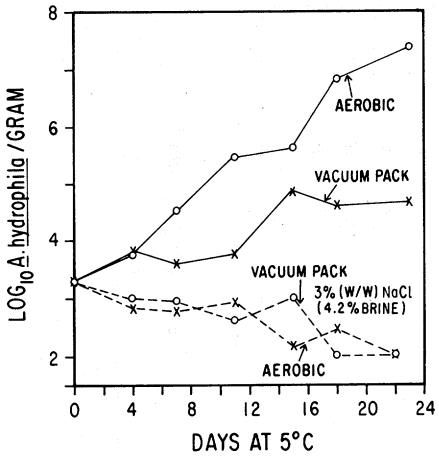

Fig. 3. Effect of packaging (aerobic vs. vacuum packaged) and NaCl (3%, dashed line) on the response of *A. hydrophila* K144 in ground pork (starting pH 6.40) held at 5 ° C.

declined, viable *A. hydrophila* also declined. This decrease of pH appears to be brought about by acid products formed by the organism as well as by developing lactic acid bacteria; at 20 days of storage, the count of lactic acid bacteria was 1.8×10^7/g and the total aerobic count was 6.5×10^8/g. The combination of $1\frac{1}{2}$% (w/w) NaCl (2.4% brine) and 1% glucose gave a intermediate response of *A. hydrophila* (compare Fig. 1b with Fig. 2b [right]). The pH of the ground pork with NaCl and glucose was starting to decline and had additional samples been taken, a further decline similar to that seen with only 1% glucose (Fig. 2a [left]) might have been seen.

Since red meats are often vacuum (anaerobically) packaged to extend the microbiological shelf life, the effect of vacuum packaging on the development of *A. hydrophila* in ground pork was studied (Fig. 3). In addition, the effect of 3% (w/w) NaCl under aerobic and vacuum packaging was evaluated. The control grew well during the course of storage (Fig. 3); in the vacuum packed ground pork, there was only a small increase in viable *A. hydrophila.* As expected, the presence of 3% (w/w) NaCl (4.2% brine) suppressed the growth of the organism. While the organism did not grow, it died off only gradually and viable cells were detected in the vacuum packed sample containing 3% NaCl after 32 days of storage (viable count of *A. hydrophila* was 2×10^2/g). Thus while not permitting growth of *A. hydrophila*, the combination of vacuum packaging and 3% NaCl was not highly lethal to the organism. In general, except for variables which caused a decline of pH to below 6.0, most parameters supported viability over an extended storage period at 5 ° C.

The background flora of various foods can influence the behavior of different pathogens. In this part of the study, the background flora of the ground pork was reduced by irradiation (150 krads in a ^{137}Cs source). This irradiated pork was then inoculated with *A. hydrophila* K144 and the influence of variables such as NaCl, glucose, and atmosphere studied (Fig. 4a and b). In Fig. 4a, the effect of back-

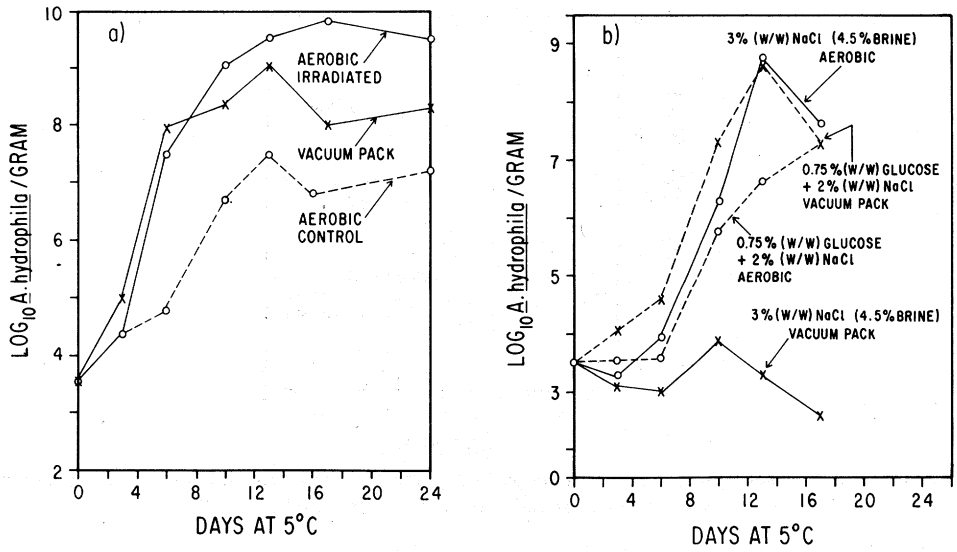

Fig. 4. Effect of background flora, NaCl, glucose and packaging on the response of *A. hydrophila* K144 in ground pork (starting pH 6.42) held at 5 °C (aerobic control is non-irradiated).

ground flora and atmosphere was studied. Moderate growth of *A. hydrophila* was seen aerobically in the presence of normal meat microflora; when the microflora is reduced by irradiation (aerobic count reduced by two logs), very good growth of *A. hydrophila* is obtained aerobically. Vacuum packed ground pork also supported moderately good growth of *A. hydrophila*. In previous experiments, a lactic microflora developed and this brought about a dramatic pH drop when glucose was added to the ground pork. In the present experiment (Fig. 4a), the microflora had inhibited the growth of *A. hydrophila* in the absence of a pH drop (in fact, the pH of the aerobic control increased from 6.4 to 7.2 during the 24 day storage period). Under limited competition (Fig. 4b), *A. hydrophila* grew in an aerobic pack containing 3% NaCl and in an aerobic pack containing 2% NaCl and 0.75% glucose (the pH remained unchanged during the storage period at 5 °C). When the ground pork was vacuum packaged, 3% NaCl inhibited growth of *A. hydrophila,* but viability again was maintained.

The effect of background flora appears to be non specific. This non specific inhibition by background flora has been observed for *Yersinia enterocolitica* (Schiemann and Olson, 1984) in milk (Stern et al., 1980) and pork (Fukushima and Gomyoda, 1986; Hanna et al., 1977). In a survey of retail fresh foods of animal origin cited previously (Palumbo et al., 1985a), *A hydrophila* was observed to increase 100–1000 fold during one week's storage at 5 °C. However, the organism seldom become the predominant organism and only once constituted as much as 10% of the total aerobic count. At the time of our food survey (Palumbo et al., 1985a), the pH of the foods was not monitored, so that an inhibition based on decreased pH can not be suggested. Except for the fresh sausage, none of the foods

had any salt added. Thus, inhibition by the natural aerobic flora of the various foods is offered as an explanation of this restricting effect.

A. hydrophila has often been observed to develop as a component of the flora of stored red meats (Blickstad and Molin, 1983; Enfors et al., 1979; Grau et al., 1985; Lee et al., 1985; Seideman et al., 1976; Simard et al., 1984). The conditions under which the organism developed varied considerably. Since these were not inoculated pack studies, it is not possible to determine if the observed development of *A. hydrophila* or lack of it is a function of the number present originally, the conditions under which the meat was stored, the microflora which developed on the meat during storage, or a combination of the three.

The factors affecting the growth of *A. hydrophila* on ground pork appear to be complex and interrelated. One can not specify one controlling variable in any experiment. Some generalizations are possible. The organism is sensitive to low pH, whether the naturally occuring low pH or caused by lactic acid bacteria acting on added glucose. Naturally occurring background microflora can inhibit *A. hydrophila* in the absence of a pH drop. Salt levels which inhibit growth did allow survival often for extended periods. Salt was not a practical means of inhibiting at pH greater than 6.5.

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